NATURE READ IN TOOTH AND FUR: NON-INVASIVE SAMPLING OF PYGMY SHREW (*SOREX MINUTUS* LINNAEUS, 1766) ON LUNDY FOR POPULATION GENETICS

by

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ABSTRACT

Britain's smallest mammal, the pygmy shrew (*Sorex minutus* Linnaeus, 1766) is the only long established terrestrial mammal on Lundy. Little is known about this species' mode of colonisation or its population structure on the island. Genetic analyses might provide useful insights in this respect, but DNA samples are difficult to obtain because pygmy shrews can be harmed by sampling methods. Two non-invasive methods for obtaining DNA sequences were tested. DNA was extracted from hair samples and from a mandible derived from a bird-pellet. A 612 base pair fragment of the cytochrome oxidase b gene was PCR amplified and sequenced. To investigate the origin of pygmy shrew on Lundy the sequences were analysed in the context of published data. The sequences are most similar to those from the U.K. mainland which supports a British origin of this population.

Keywords: *Cytochrome oxidase b, phylogeography, non-invasive sampling, hair tubes, island population, molecular ecology, small mammal*

INTRODUCTION

The pygmy shrew (*Sorex minutus*) is the smallest mammal species in the British Isles, is found throughout the British and Irish mainlands at all altitudes and is widespread on most small and large coastal islands (Churchfield, 1990; Yalden, 1999). It is the only terrestrial mammal long established on Lundy (Linn, 1997). Although the Lundy population has been subjected to various population surveys (Bull & Parker, 1997; Marshall *et al.*, 2009), little is known about its origin, genetic structure and divergence.

Island colonisation by small mammals is an area of widespread interest (Montgomery *et al.*, 2014; Nowack & Dausmann, 2015). When the juxtaposition of mainland Britain and continental Europe is considered there are numerous routes via which pygmy shrew may have returned to Britain and Ireland after the Last Glacial Maximum (LGM). Phylogeographic studies reveal a genetic structure referred to as a 'celtic fringe' with genetically distinct 'northern' and 'western' lineages of pygmy

shrew (Mascheretti *et al.*, 2003; Searle *et al.*, 2009). Based on these observations, McDevitt *et al*. (2011) presented four potential colonisation scenarios. One scenario postulates that mainland Britain was re-colonised twice, with an initial 'wave' of colonising animals displaced by a second wave, both from continental Europe. A second scenario assumes that there have been multiple introductions of pygmy shrew at different points throughout Britain and Ireland from central Europe and the Iberian Peninsula. Based upon their data, McDevitt *et al*. (2011) suggested that the first postulate was most likely.

'Western' haplotypes dominate large islands (Isle of Wight, Anglesey) and archipelagos (the Hebrides and the Orkney islands) off the coast of mainland Britain (McDevitt *et. al*., 2011). This observation has been used to suggest that their current populations result from introductions by humans during the Neolithic period (Mascheretti *et al.*, 2003; Searle *et al.*, 2009).

The Lundy population was investigated in order to determine whether it fits the 'celtic fringe' hypothesis or whether as a very small and isolated off-shore island, different influences have been in operation. Because pygmy shrews can easily be harmed by trapping (Bull and Parker, 1997; Marshall *et al*., 2009), non-invasive methods (Taberlet, 1999) of sampling were used to obtain DNA. Hair samples were collected using a modified hair tube method (Pocock & Bell, 2011), from a taxidermic collection and a voucher specimen. In addition, the usability of a pygmy shrew mandible found in a bird pellet was tested.

METHODS

Sample collection

In June 2014, transects (27 metres long) of hair tubes (12.5mm aperture, Faunagoo™ wafers as hair entrapment surfaces) were placed across Lundy in the following habitats: wet heathland, dry (waved) heathland, vegetated combes and grasslands. Sites were chosen according to habitat suitability for pygmy shrew as described in Harris & Yalden (2008). One baited hair tube was placed at ground level every 3 metres (totalling 10 on each transect). Hair tubes were secured using a tent peg to prevent displacement by birds or grazing mammals and remained in the field for a minimum of one night. Plate 1 shows a typical example of this arrangement. Wafers were checked for pygmy shrew hairs using a ×10 hand lens. If positive signs were found then wafers were placed into a transparent bag for later microscopic examination. Pygmy shrew guard hairs were distinguished by their length (2mm) and characteristic 'zig-zag' profile (Pocock & Jennings, 2006; Teerink, 1991).

Hair samples were kept on the sticky wafers within zip-lock bags at room temperature before undergoing DNA extraction. Additional samples included a pygmy shrew mandible found within a bird pellet collected on Lundy in June 2014. In addition, a Lundy pygmy shrew tissue sample from 1965 was obtained from a personal archive (*pers. comm.* Morris, 2015). Positive controls for amplification were collected from bank vole (*Myodes glareolus*) and field vole (*Microtus agrestis*) obtained via domestic cat kills on the British mainland. A taxidermic specimen (Middlesex University, Environmental Health) was used as a further positive control for pygmy shrew.

Plate 1: A hair tube *in situ* near the Old Light on the West Sideland, Lundy. Hair tubes were secured using tent pegs to prevent them being moved and transects marked using bamboo flags to aid surveyor identification

DNA extraction, PCR amplification and Sanger sequencing

DNA was extracted using the DNeasy Blood & Tissue Kit (Qiagen, Valencia, CA, USA) using a user-developed protocol available from the Qiagen website (Qiagen, 2006). Part of the cytochrome oxidase B (*CytB*) gene was amplified using primers Vega1F (Vega *et al.*, 2010) and Searle-R (Searle *et al.* 2009). PCR amplification of the 612 bp target region was carried out in a 25µl final volume containing: 1µl each primer (10µM), 5µl DNA, 8.5µl nuclease-free H_2O and 12.5µl Master Mix (Promega) with cycling conditions: 94°C for 5 minutes, 35 cycles at 92°C for 30 seconds, 53°C for 30s and 72°C for 30s and a final elongation step at 72°C for 5 minutes using a TC-512 thermal cycler (Techne). PCR products were sent to the NHM London sequence facility and sequenced in both directions on an Applied Biosystems 3730xl DNA analyser.

Sequence Analyses

Geneious (Kearse *et al.*, 2012) was used to assemble sequence reads and to remove trailing primer sequences. Pygmy shrew consensus sequences were aligned to publicly available data (McDevitt *et al.*, 2011; using MAFFT (E-INS-I algorithm) Katoh *et al.*, 2002). Flanking ends were trimmed to the start and ends of the targeted region. Sequences that were shorter than the targeted region were removed from the alignment, resulting in a final alignment of 323 sequences. Haplotype networks were constructed in PopART (Leigh & Bryant, 2015) using the method of TCS (Clement *et al.*, 2002) to infer relationships among samples. The *CytB* sequences were deposited in GenBank (accession numbers: KT357614, KT357615 and KT357616).

RESULTS

Cytochrome oxidase B sequences were obtained for three Lundy pygmy shrew samples and combined with publicly available data. Of those sequences included in the study, the Lundy sequences (derived from a hair sample, archived tissue and mandible) showed a unique T-C synonymous substitution at position 211. Two different Lundy haplotypes were obtained, with the 2014 samples being identical. Haplotype networks were constructed. The PopART statistical parsimony network is shown in Figure 1, which shows the juxtaposition of the two haplotypes obtained in the context of data of McDevitt *et al.* (2011). It can be seen that the Lundy sequences are genetically distinct from any other European sequences included in this analysis. They are most similar to those from south-west Wales.

DISCUSSION

Non-invasive sampling permits genetic analyses of animals without having to capture them (Taberlet *et al.*, 1999). This is particularly significant when studying vulnerable, elusive and protected species such as the pygmy shrew (Churchfield, 1990). In this study non-invasive methods were used to examine the pygmy shrew population on Lundy. All three sample types produced DNA sequences, including the pygmy shrew mandible and a 50 year old tissue sample. Lundy sequences were shown to be distinct, but show high similarity to sequences obtained from coastal regions of Pembrokeshire and Carmarthenshire. This suggests that Lundy was not colonised from the Iberian peninsula (as has been hypothesised in previous studies for some U.K. populations; McDevitt *et al.*, 2011), but from a mainland Britain source population.

If the Lundy pygmy shrew originates from the periphery of the British mainland and not from the Iberian peninsula it is likely to have either migrated onto the island via a land bridge some time after the LGM, or to have been unwittingly introduced from the British mainland by people, possibly as early as the Mesolithic. If the pygmy shrew arrived on Lundy without the aid of people this was most likely the result of the species' high mobility, enabling it to colonise newly exposed territory more rapidly than less mobile species (Linn, 1997).

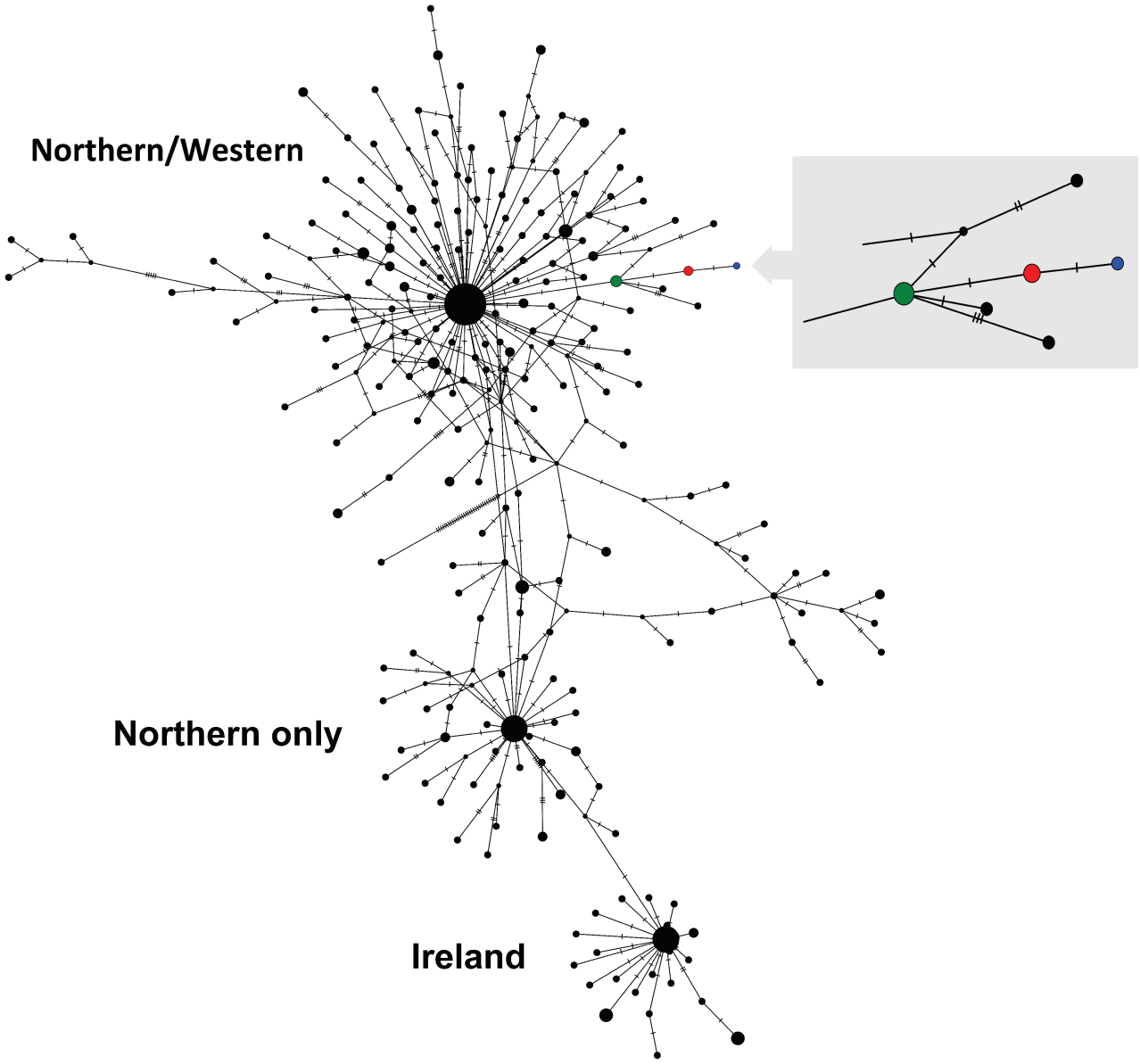

Figure 1: Statistical parsimony network showing the relationships between CytB sequences. The majority of samples are from McDevitt *et al.* (2011). Identical haplotypes have been grouped together and frequencies are represented by circle size. Mandible and hair tube samples are highlighted with a red circle, archived material with a blue circle and south-west Wales haplotypes with a green circle

Such newly exposed land bridge surfaces are likely to have been waterlogged acidic peats that acted as filters to prevent common shrews from reaching Lundy (Yalden, 1981). Thus, by foraging close to the retreating ice it may have reached outlying regions before other, less hardy species, and before rising sea levels cut off the areas of land on which they found themselves. Other fauna such as Lundy cabbage flea beetle (*Psylliodes luridipennis*) and flora such as the Lundy cabbage

(*Coincya wrightii*) have also naturally colonised the island using this route (Compton *et al*., 2007). However, it should be stressed that it is not possible to differentiate between the hypotheses that the pygmy shrew colonised Lundy via a land bridge or by another means (such as human agency) after rising sea levels made it an island.

The Lundy sequences are distinct and are not shared with any other recorded European population. In the context of McDevitt *et al*. (2011) data, they are located at the periphery of the northern/western haplotype cluster, which agrees with previous trends. More sampling of West coast maritime environments is required to confirm whether they are unique.

This study confirms that hair tubes offer a viable alternative to Longworth or other live trapping methods for collecting pygmy shrew hair samples in protected areas and can be usefully augmented via archive and non-traditional sample sources, such as in this case from bird pellet content. Sequences obtained suggest that the Lundy pygmy shrew forms a distinct population, as might be expected on such a small, isolated island on which evolutionary processes such as founder principle and genetic drift are likely to exert a profound influence. As a consequence this terrestrial, long established population forms a valuable resource for future evolutionary studies.

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REFERENCES

Bull, S.B. & Parker, R.D. (1997). A study of the Pygmy Shrew (*Sorex minutus*) on Lundy. *Annual Report of the Lundy Field Society 1996,* **47**, 50-57

Churchfield, S. (1990). *The natural history of shrews.* London*:* Christopher Helm Publishers Ltd

Clement, M., Snell, Q., Walker, P., Posada, D. & Crandall, K. (2002). TCS: Estimating gene genealogies. *Parallel and Distributed Processing Symposium, International Proceedings,* 2, 184

Compton, S.G., Craven, J.C., Key, R.S. & Key, R.J. (2007) Lundy Cabbage: Past, Present and Future. In: George, J. ed, *Lundy Studies.* Bideford: Lundy Field Society, 59-69

Harris, S. & Yalden, D.W. (2004). An integrated monitoring programme for terrestrial mammals in Britain. *Mammal Review*, **34**(102), 157-167

Katoh, K., Misawa, K., Kuma, K.I. & Miyata, T. (2002). MAFFT: a novel method for rapid multiple sequence alignment based on fast Fourier transform. *Nucleic acids research*, **30**(14), 3059-3066

Kearse, M., Moir, R., Wilson, A., Stones-Havas, S., Cheung, M., Sturrock, S. & Drummond, A. (2012). Geneious Basic: an integrated and extendable desktop software platform for the organization and analysis of sequence data. *Bioinformatics*, **28**(12), 1647-1649

Leigh, J.W. & Bryant, D. (2015). PopArt: full-feature software for haplotype network construction. *Methods in Ecology and Evolution*, **6**(9), 1110-1116

Linn, I. (1997). Wild mammals of Lundy. In: Irving, R.A., Schofield, A.J. & Webster, C.J. (eds.) *Island Studies,, Fifty Years of the Lundy Field Society*. Bideford: Lundy Field Society, 107-117

Marshall, K., Blet-Charaudeau, C., Leaver, L. and Lea, S.E.G. (2009). Pygmy Shrews *Sorex minutus* on Lundy: Preliminary Data on Distribution and Effectiveness on trapping methods. *Annual Report of the Lundy Field Society 2008*, 58, 86-88

Mascheretti, S., Rogatcheva, M.B., Gündüz, İ., Fredga, K. & Searle, J.B. (2003). How did pygmy shrews colonise Ireland? Clues from a phylogenetic analysis of mitochondrial cytochrome b sequences. *Proceedings of the Royal Society of London B: Biological Sciences,* **270**(1524), 1593-1599

McDevitt, A.D., Vega, R., Rambau, R.V., Yannic, G., Herman, J.S., Hayden, T.J. & Searle, J.B. (2011). Colonization of Ireland: revisiting 'the pygmy shrew syndrome' using mitochondrial, Y chromosomal and microsatellite markers. *Heredity,* **107**(6), 548-557

Montgomery, W.I., Provan, J., McCabe, A.M. & Yalden, D.W. (2014) Origin of British and Irish mammals: disparate post-glacial colonisation and species introductions. *Quaternary Science Reviews*, 98, 144-165

Nowack, J. & Dausmann, K.H. (2015). Can heterothermy facilitate the colonization of new habitats?. *Mammal Review*, **45**(2), 117-127

Pocock, M.J.O. & Bell, S.C. (2011). Hair tubes for estimating site occupancy and activity-density of *Sorex minutus*. *Mammalian Biology*, **76**(4), 445-450

Pocock, M.J.O. & Jennings, N. (2006). Use of hair tubes to survey for shrews: new methods for identification and quantification of abundance. *Mammal Review*, **36**(4), 299-308

Qiagen (2006) *Purification of total DNA from nails, hair, or feathers using the DNeasy® Blood & Tissue Kit - (EN)* (2006) Available at: https://www.qiagen.com/gb/resources/resourcedetail?id=a5a065dc-e287-4a61-b917-9792e25ab42f&lang=en (accessed Tuesday 11 November 2014)

Searle, J.B., Kotlík, P., Rambau, R.V., Marková, S., Herman, J.S. & McDevitt, A.D. (2009). The Celtic fringe of Britain: insights from small mammal phylogeography. *Proceedings of the Royal Society B: Biological Sciences*, 276, 4287-4294

Taberlet, P., Waits, L.P. & Luikart, G. (1999). Noninvasive genetic sampling: look before you leap. *Trends in Ecology & Evolution*, **14**(8), 323-327

Teerink, B. (1991). *Hair of West-European mammals: atlas and identification key.* Cambridge: Cambridge University Press

Vega, R. (2010). *Phylogeographic and morphometric studies on the Eurasian pygmy shrew Sorex minutus: insights into its evolutionary history and postglacial colonisation in Europe.* PhD thesis. University of York. Available at: http://etheses.whiterose.ac.uk/1039/ (accessed 2 March 2015)

Yalden, D.W. (1981). The occurrence of the Pigmy shrew *Sorex minutus* on moorland, and the implications for its presence in Ireland. *Journal of Zoology*, **195**(2), 147-156

Yalden, D.W. (1999). *The history of British mammals*. London: A. & C. Black Publishers Ltd.